DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

Date DEC 15 1995

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Gen-Probe® Amplified *Mycobacterium tuberculosis* Direct Test - ACTION

To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA supplement.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Susan Alpert, Ph.D., M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved _____ Disapproved _____ Date _____

Prepared by Roxanne Shively, CDRH, HFZ-440, 12/13/95, 594-2096

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[DOCKET NO. _____]

Gen-Probe®, Inc.; PREMARKET APPROVAL of Gen-Probe® Amplified *Mycobacterium tuberculosis* Direct Test

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Gen-Probe®, Inc., San Diego, CA, for premarket approval, under the Federal Food, Drug, and Cosmetic Act (the act), of the Gen-Probe® Amplified *Mycobacterium tuberculosis* Direct Test. After reviewing the recommendation of the Microbiology Devices Panel, FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter on December 15, 1995, of the approval of the application.

DATES: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESSES: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review, to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Sharon L. Hansen, Ph.D.,

Center for Devices and Radiological Health (HFZ-440),

Food and Drug Administration,

2098 Gaither Rd.,

Rockville, MD 20850,

301-594-2096.

SUPPLEMENTARY INFORMATION: On July 11, 1994, Gen-Probe®, Inc., San Diego, CA, 92121, submitted to CDRH an application for premarket approval of the Gen-Probe® Amplified *Mycobacterium tuberculosis* Direct Test (MTD). The device is a target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid fast bacilli (AFB) smear positive concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates), or tracheal aspirates. The MTD test is to be used as an adjunctive test for evaluating AFB smear positive concentrated sediments prepared using NALC-NaOH digestion-decontamination of respiratory specimens from untreated patients suspected of having tuberculosis. Patients who have received no anti-tuberculous therapy, less than 7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated

with this test. The MTD test should be performed only in laboratories proficient in the culture and identification of *M. Tuberculosis* (Level II and III, or extent 3 and 4). The MTD should always be performed in conjunction with mycobacterial culture. On May 2, 1995, the Microbiology Devices Panel of the Medical Devices Advisory Committee, an FDA advisory panel, reviewed and recommended approval of the application.

On December 15, 1995, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

Opportunity for Administrative Review

Section 515(d)(3) of the act, (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act, for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices

and procedures regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h), (21 U.S.C. 360e(d), 360j(h))) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated: _____.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

DEC 15 1995

Mr. David E. Dunn
Gen-Probe, Inc.
9880 Campus Point Drive
San Diego, California 92121

Re: P940034
Gen-Probe® Amplified *Mycobacterium tuberculosis* Direct Test
Filed: July 11, 1994
Amended: November 18, November 29, and December 27, 1994; February 7, March 24, March 28, April 24, May 30, September 7, November 6 and December 15, 1995.

Dear Mr. Dunn:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Gen-Probe® Amplified *Mycobacterium tuberculosis* Direct Test (MTD). The device is a target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid fast bacilli (AFB) smear positive concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates), or tracheal aspirates. The MTD test is to be used as an adjunctive test for evaluating AFB smear positive concentrated sediments prepared using NALC-NaOH digestion-decontamination of respiratory specimens from untreated patients suspected of having tuberculosis. Patients who have received no anti-tuberculous therapy, less than 7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated with this test. The MTD test should be performed only in laboratories proficient in the culture and identification of *M. tuberculosis* (Level II and III, or extent 3 and 4). The MTD should always be performed in conjunction with mycobacterial culture.

We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution, and use of this device are restricted to prescription use in accordance with 21 CFR 801.109 within the meaning of section 520(e) of the Federal Food, Drug, and Cosmetic Act (the act) under the authority of section 515(d)(1)(B)(ii) of the act. FDA has also determined that to ensure the safe and effective use of the device that the device is further restricted within the meaning of section 520(e) under the authority of section 515(d)(1)(B)(ii), (1) insofar as the labeling specify the requirements that apply to the training of practitioners who may use the device as approved in this order, (2) insofar as the

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labeling specify the requirements that apply to laboratory facilities where the device is to be used as approved, and (3) insofar as the sale, distribution, and use must not violate sections 502(q) and (r) of the act.

In addition to the postapproval requirements in the enclosure, postapproval reports must include the following information and data to be collected and submitted in accordance with a protocol reviewed by FDA:

1. Results of testing specimens with the inhibition control recommended in the package insert. These results will be documented as part of new customer auditing for six months.

2. Results of contamination monitoring performed at least monthly and at other times, as needed to troubleshoot aberrant controls, at each laboratory using the device during the new customer auditing.

3. An evaluation of the effects of excess NaOH exposure on detection of *M. tuberculosis* complex rRNA using the MTD.

4. An evaluation of the effects of using bovine serum albumin, phosphate buffered saline, or sterile water as a resuspension fluid for the NALC-NaOH prepared sediments on detection of *M. tuberculosis* complex rRNA using the MTD.

5. An evaluation of the reproducibility of MTD results with fresh sediments and frozen sediments.

6. Results of proficiency testing required of new users (laboratories).

Expiration dating for this device has been established and approved at six months under the storage conditions recommended in the package insert.

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to

this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Sharon L. Hansen, Ph.D. at (301) 594-2096.

Sincerely yours,

Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

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A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the **addition** of, but **not the replacement** of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. **This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.**

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant**(s)** involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

(a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

(b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mixup of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984, and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to FDA whenever they receive or otherwise became aware of information that reasonably suggests that one of its marketed devices

(1) may have caused or contributed to a death or serious injury or

(2) has malfunctioned and that the device or any other device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for this PMA, you shall submit the appropriate reports required by the MDR Regulation and identified with the PMA reference number to the following office:

Division of Surveillance Systems (HFZ-531)
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, 340
Rockville, Maryland 20850
Telephone (301) 594-2735

Events included in periodic reports to the PMA that have also been reported under the MDR Regulation must be so identified in the periodic report to the PMA to prevent duplicative entry into FDA information systems.

Copies of the MDR Regulation and an FDA publication entitled, "An Overview of the Medical Device Reporting Regulation," are available by written request to the address below or by telephoning 1-800-638-2041.

Division of Small Manufacturers Assistance (HFZ-220)
Center for Devices and Radiological Health
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. General Information

Device Generic Name: Target Amplification Test for the Direct Detection of Mycobacterium Tuberculosis

Device Trade Name: Gen-Probe® Amplified Mycobacterium Tuberculosis Direct (MTD) Test

Applicant's Name and Address:

Gen-Probe Incorporated
9880 Campus Point Drive
San Diego, CA 92121-1589

Premarket Approval Application (PMA) Number: P940034

Date of Panel Recommendation: May 2, 1995

Date of Notice of Approval to the Applicant: December 15, 1995.

II. Indications for Use

The Gen-Probe Amplified Mycobacterium Tuberculosis Direct (MTD) Test is a target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid fast bacilli (AFB) smear positive sediments prepared from sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates), or tracheal aspirates.

The MTD test is to be used as an adjunctive test for evaluating AFB smear positive sediments prepared using NALC-NaOH digestion-decontamination of respiratory specimens from untreated patients suspected of having tuberculosis. Patients who have received no anti-tuberculous therapy, less than 7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated with this test. The MTD test should be performed only in laboratories proficient in the culture and identification of *M. tuberculosis* (Level II and III, or extent 3 and 4 laboratories).[1] The MTD should always be performed in conjunction with mycobacterial culture.

Contraindications:

There are no known contraindications for the MTD Test.

Warnings and Precautions:

Warnings and Precautions for use of the device are stated in

the attached product labeling. (Attachment A)

A. Background

1. *Mycobacterium tuberculosis* complex

M. tuberculosis complex includes the species *M. bovis*, *M. microti*, and *M. africanum*. However, *M. microti* infects only animals, *M. bovis* is uncommonly transmitted from infected animals to humans, and *M. africanum* causes pulmonary disease in humans in tropical Africa.[9] *M. tuberculosis* is by far the most common member of the complex that is responsible for human disease worldwide. Nontuberculous mycobacteria (Mycobacteria other than tuberculosis or MOTT) include *M. avium* complex (MAC) and other organisms that may also cause disease in humans.

M. tuberculosis complex and MOTT are presumptively identified in slides made from clinical specimens by their acid fast nature. All mycobacteria have cell walls with a high lipid content that prevents easy decolorization once stained (acid fastness). Growth rates are slow to very slow, with some species requiring supplementation for laboratory culture.[7] Definitive identification of *M. tuberculosis* and MOTT requires identification by traditional methods (observation of growth rate, colonial morphology, pigmentation and biochemical profiles), chromatographic analysis of lipid composition (thin layer chromatography, capillary gas chromatography, or high pressure liquid chromatography), or hybridization assays with specific nucleic acid probes.

M. tuberculosis complex organisms are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory. The infective dose for humans is low (ID_{50} <10 bacilli). Biosafety Level 2 practices are required for activities at the American Thoracic Society (ATS) laboratory Level I (preparation of AFB smears, collection and transport of mycobacterial specimens for culture). Biosafety Level 3 practices are required for laboratory activities of ATS levels II (isolation and identification of *M. tuberculosis*) and III (additionally performing susceptibility testing of *M. tuberculosis* isolates and identification of MOTT).[7]

2. Tuberculosis

Tuberculosis is a bacterial disease caused by organisms of the *Mycobacterium tuberculosis* complex; it is transmitted primarily by airborne droplet nuclei from individuals with pulmonary or laryngeal tuberculosis.[8]

Tuberculosis (TB) can occur in any organ of the body, but only 5 to 15 percent of infected individuals will develop active disease within 2 years of primary infection. Pulmonary TB is still the primary manifestation in infected individuals who develop disease, but the incidence of extrapulmonary TB has progressively increased.[10] HIV-infected individuals who develop active TB have a high rate of extrapulmonary disease. HIV-positive patients with low CD4 counts tend to have radiographic presentations markedly different from the classical upper lobe cavitary disease of immunocompetent patients. Clinical disease due to reactivation of dormant TB is influenced by predisposing factors such as AIDS, malignancy, silicosis, immunosuppressive therapy, malnourishment, and other risk factors.[10]

Population groups in the United States that are at increased risk for infection with *M. tuberculosis* include medically underserved, low-income populations, immigrants from countries with a high prevalence of TB, and residents of long-term-care and correctional facilities. Those at increased risk of developing disease following infection include individuals with HIV infection; close contacts of infectious cases; children less than 5 years old; patients with renal failure, silicosis, and diabetes mellitus; and individuals receiving treatment with immunosuppressive medications.[8]

The initial treatment of TB includes multiple antimicrobial agents, since administration of a single drug often leads to the development of resistance. *M. tuberculosis* becomes drug resistant through random, spontaneous genetic mutation. Susceptibility testing of the first isolate from all patients should be done to provide the physician a basis for therapeutic management, to identify emerging drug resistance, and to help monitor control efforts in area where resistance is established. If culture positive sputum continues after three months of therapy, susceptibility testing should be repeated. During the first week of therapy, few patients convert from culture positive to culture negative.[5] Thereafter, patients responding to therapy will have significant

reductions in organism loads and become culture negative. The time course until a patient becomes noninfectious is influenced by initial organism load, the presence of a drug-resistant strain, and the severity of coughing. Organism load reduction can be monitored with AFB smears; culturing is used to monitor bacteriologic sputum conversion, to assess response to therapy, and to monitor the emergence of resistant strains.[4]

After uniform national reporting of TB began in 1953, the number of cases reported annually declined steadily until 1985. Since that time TB has reemerged as a serious public health problem.[8] In addition, the development of multi-drug resistant strains of *M. tuberculosis* has become a major concern. Factors contributing to the increase in TB morbidity in the United States include an increase in foreign-born cases, the HIV/AIDS epidemic, and increased active TB transmission in higher risk populations.[2]

III. Device Description

The MTD test utilizes Transcription Mediated Amplification (TMA) and the Hybridization Protection Assay (HPA™, Gen-Probe, Inc.) to qualitatively detect *M. tuberculosis* complex ribosomal ribonucleic acid (rRNA) in respiratory specimens. Initially, 50 µL of processed (digested and decontaminated) respiratory specimen sediment is sonicated to release cellular nucleic acids. Each intact *M. tuberculosis* cell contains approximately 2000 copies of rRNA. The MTD test uses TMA to amplify *M. tuberculosis* rRNA in clinical specimens to levels detectable with HPA. TMA is an RNA transcription-dependent amplification technology, in which RNA strands serve as templates for the synthesis of DNA intermediates. These DNA intermediates are then used for the transcription of multiple copies of RNA amplicon. The RNA amplicon can then serve as templates for further synthesis of DNA intermediates, which in turn are used for further transcription of additional copies of RNA amplicon.

In the HPA step of the MTD, *M. tuberculosis* complex-specific rRNA sequences in amplicons are detected by hybridization with a chemiluminescent-labeled DNA probe (Detector Probe) that is complementary to sequences in the amplicon. A key aspect of HPA is the steric protection of the acridinium ester chemiluminescent label that is linked to the Detection Probe from a hydrolysis reaction that destroys the chemiluminescence of the label. This protection occurs only when the Detection Probe hybridizes with the complementary amplicon sequence. When a selection reagent (containing a high pH buffer) is added to a solution containing a mixture of hybridized and

unhybridized Detection Probe, only hybridized probe will retain chemiluminescent properties. Following this "differential hydrolysis" step, Detection Reagents are added to the solution and the acridinium ester molecules that are attached to the hybridized Detection Probes emit photons. The photons emitted (chemiluminescence) are measured with a luminometer (LEADER™, GenProbe, Inc.) as Relative Light Units (RLU). The presence or absence of the target rRNA is determined by the level of RLU measured (positive = ≥30,000 RLU). The magnitude of the RLU reading is not indicative of the numbers of *M. tuberculosis* organisms present in the specimen, as saturated levels are obtained above 60 and up to 500 copies of *M. tuberculosis* rRNA (equivalent to 0.05 to 0.25 cells *M. tuberculosis*).

The MTD test will detect rRNA from both cultivable and non-cultivable organisms within the *M. tuberculosis* complex and enables detection of *M. tuberculosis* complex rRNA within 4 to 5 hours after beginning the test procedure. The MTD test cannot ascertain drug susceptibility and cannot differentiate between viable and non-viable cells. MTD results, therefore, cannot be used for specimens from treated patients, since nonculturable organisms may be shed in the respiratory secretions during antituberculous therapy; a positive MTD result will not assess organism viability and a negative result would not monitor response to therapy.

The MTD device kit includes a Hybridization Positive Control (HP) and an Hybridization Negative Control (HN). The HP is comprised of approximately 3 X 10^9 copies (equivalent to 1.5 X 10^6 organisms) of a synthetic DNA oligonucleotide that is complementary to the probe, and monitors the hybridization portion of the device. The HN is essentially a reagent blank that monitors background chemiluminescence and contamination. Additional controls (*M. tuberculosis* complex and MOTT cell suspensions prepared from cultures) that monitor the amplification and hybridization portions of the assay must be prepared and evaluated for acceptability by each laboratory performing the assay.

Results from the MTD test may be affected by inhibitory substances (exogenous or endogenous) contained in clinical specimens. Amplicon from previous MTD testing or *M. tuberculosis* in the laboratory may contaminate reagents, laboratory surfaces, and equipment resulting in false positive MTD results. Amplification and hybridization are performed in the same tube, thereby avoiding a transfer procedure that could be an added source of contamination. Nevertheless, the MTD must be performed with appropriate precautions and strict adherence to the specified procedure.

IV. Alternative Practices and Procedures

Tuberculin skin testing, radiography, assessment of physical findings, and identification of risk factors are used to determine patients with a high index of suspicion that TB may be present. AFB smears and cultures of clinical material are necessary to establish a definitive diagnosis of TB although a strong presumptive diagnosis may be made on radiographic findings when the patterns are typical.[1]

Definitive diagnosis of mycobacterial disease (except leprosy), including TB, requires growth of the microorganism. Although patients will be initially treated with a predetermined therapeutic regimen, cultures are also required for susceptibility testing to confirm the anticipated effectiveness of treatment. Culture for AFB is usually performed by inoculating several media with decontaminated sediment and incubating for up to 8 weeks. Conventional culture methodologies can detect *M. tuberculosis* growth as early as 1 week, but may take up to 8 weeks. Radiometric liquid culture, requires an average of 13 days to final culture result.[6] Current recommendations from CDC are to inoculate both a liquid medium and a solid medium.[8] After recovery of mycobacteria from culture media, identification of *M. tuberculosis* may be done by conventional biochemical testing, analysis of lipid content, or hybridization with specific DNA probes.

The American Thoracic Society (ATS), in collaboration with CDC, provides a classification scheme for TB that is based on pathogenesis and current treatment recommendations.[1] Patients with clinical suspicion of TB or positive AFB smears are reported to local health departments for appropriate public health management (including contact investigations).[8] Final species identification and susceptibility results from positive cultures are also reported to the health department.

V. Marketing History

The MTD test has been marketed in Australia, Austria, Canada, Denmark, Finland, France, Germany, Greece, Hong Kong, Italy, The Netherlands, New Zealand, Norway, South Africa, Spain, Sweden, Switzerland, Taiwan, the United Kingdom, and Japan.

VI. Potential Adverse Effects of the Device on Health

Prompt diagnosis of TB is critical, both to initiate appropriate therapy and to institute measures to prevent further exposures and spread of the disease to uninfected individuals, both in the community and health care facilities. A false positive result could lead to misdiagnosing a patient's medical status, resulting in the administration of unnecessary therapy and/or placing a patient in unwarranted

unnecessary therapy and/or placing a patient in unwarranted isolation; additionally, the patient would be reported to the local health department for public health management, and contact investigations initiated. A false negative result could delay the correct diagnosis of TB and initiation of appropriate therapy. Delayed diagnosis can result in rapidly progressive disease, especially in HIV-positive patients and patients infected with multi-drug resistant strains of *M. tuberculosis*. In addition, the potentially infectious patient might not be placed into isolation until the results of culture were available.

False positive or false negative results can be caused by specimen inhibition, insufficient mixing, procedural deviations, use of the test by unqualified personnel, inappropriate test result reporting, interfering substances, presence of cross-reacting species in the specimen, presence of high numbers of MOTT, presence of low numbers of *M. tuberculosis* complex in the specimen, procedural errors, carryover contamination, sample misidentification, or transcription errors. *Mycobacterium celatum* and *M. terrae*-like organisms that have only one or two rRNA mismatches to the probes used in the MTD test will react, causing false positive results.

VII. Summary of Studies

A. Analytical Studies

1. Establishment of the Cutoff Value and Validation

A total of 291 respiratory specimens negative by culture for *M. tuberculosis* and 134 specimens positive by culture for *M. tuberculosis* (including both AFB smear positive and negative samples) were tested with the MTD to determine the best cutoff value for defining specimen positivity/negativity. The cutoff was selected based on Receiver Operator Characteristic (ROC) curve analysis of the data and similar analyses. The ROC curve showed that a cutoff value of 30,000 RLU represented the best balance between sensitivity (85.4 percent) and specificity (98.9 percent).

2. Specificity of the MTD Test

Specificity of the MTD test was assessed using bacteria, fungi, and viruses. For bacteria and fungi, specificity testing included 160 strains (151 species from 58 genera) of closely related mycobacteria Table 1a), other organisms causing lower respiratory disease (Table 1b), and normal respiratory flora (Table 1c), or organisms

representing a cross-section of phylogeny (Table 1d). Typed strains were obtained from the American Type Culture Collection (ATCC), and 5 isolates were obtained from clinical laboratories. Lysates prepared from actively growing cultures (or rRNA in 4 cases) were evaluated in the MTD test according to the Test Procedure. Approximately 2-4 x 10^6 colony forming units (CFU) per reaction were tested. *M. tuberculosis A and V, M. bovis, M. africanum, M. bovis BCG,* and *M. microti* were detected at levels greater than 2,000,000 RLU. *Mycobacterium celatum* and an *M. terrae*-like strain also yielded MTD test values at levels greater than 2,000,000 RLU. Positive results (falsely positive) were obtained if *M. celatum* or *M. terrae*-like organisms were present at >30 CFU per test. No other organisms tested resulted in RLU values above the MTD cutoff (30,000 RLU).

Table 1a: MTD Specificity - Mycobacterial species

Organism	ATCC *Number*	RLU Value
Mycobacterium acapulcensis	14473	2216
Mycobacterium africanum	25420	>2,000,000
Mycobacterium agri	27406	2606
Mycobacterium aichiense	27280	4712
Mycobacterium asiaticum	25276	12313
Mycobacterium aurum	23366	10037
Mycobacterium austroafricanum	33464	3336
Mycobacterium avium	25291	5084
Mycobacterium bovis	19210	>2,000,000
Mycobacterium bovis BCG	35734	2167540
Mycobacterium celatum	*	>2,000,000**
Mycobacterium chelonae	14472	2226
Mycobacterium chelonae chemovar niacinogenes	35750	3074
Mycobacterium chelonae subsp. abscessus	19977	2683
Mycobacterium chelonae subsp. chelonae	35752	3311
Mycobacterium chitae	19627	4206
Mycobacterium chubuense	27278	3364
Mycobacterium diernhoferi	19340	2985
Mycobacterium duvalii	43910	2429
Mycobacterium engbaekii	27353	3872
Mycobacterium fallax	35219	4087
Mycobacterium farcinogenes	35753	3431
Mycobacterium flavescens	14474	2491
Mycobacterium fortuitum	6841	3153
Mycobacterium fortuitum subsp. acetamidolyticum	35931	3487
Mycobacterium gadium	27726	5607
Mycobacterium gallinarum	19710	2381
Mycobacterium gastri	15754	15141
Mycobacterium gilvum	43909	3320
Mycobacterium gordonae	14470	5565
Mycobacterium haemophilum	29584	2577
Mycobacterium intracellulare	13950	3404
Mycobacterium kansasii	12478	9950
Mycobacterium komossense	33013	3403
Mycobacterium lactis	27356	3589
Mycobacterium malmoense	29571	3228

Mycobacterium marinum	927	10983
Mycobacterium microti	19422	>2,000,000
Mycobacterium moriokaense	43059	5936
Mycoabcterium neoaurum	25795	3657
Mycobacterium nonchromogenicum	19530	3093
Mycobacterium obuense	27023	2727
Mycobacterium parafortuitum	19686	2958
Mycobacterium petroleophilum	21497	2857
Mycobacterium phlei	11758	2304
Mycobacterium porcinum	33776	2889
Mycobacterium poriferae	35087	3882
Mycobacterium pulveris	35154	2518
Mycobacterium rhodesiae	27024	3778
Mycobacterium scrofulaceum	19981	9943
Mycobacterium shimoidei	27962	4186
Mycobacterium simiae	25275	4346
Mycobacterium smegmatis	14468	2716
Mycobacterium sphagni	33027	2848
Mycobacterium szulgai	35799	3542
Mycobacterium terrae	15755	4889
Mycobacterium terrae-like	*	>2,000,000
Mycobacterium thermoresistibile	19527	2891
Mycobacterium tokaiense	27282	3748
Mycobacterium triviale	23292	2744
Mycobacterium tuberculosis A	25177	1734753
Mycobacterium tuberculosis V	27294	>2,000,000
Mycobvacterium ulcerans	19423	2314
Mycobacterium vaccae	15483	3041
Mycobacterium valentiae	29356	3036
Mycobacterium xenopi	19250	3334

* Clinical Isolate
** These organisms were clinical isolates tested at 30,00 CFU per test.

Table 1b: MTD Specificity - Lower Respiratory Tract Organisms

Organism	ATCC Number	RLU Value
Blastomyces dermatitidis	26199	3029
Chlamydia pneumoniae (50 ng rRNA)	VR1356	2764
Coccidioides immitis	28868	3556
Cryptococcus neoformans	32045	3579
Haemophilus influenzae	19418	2213
Haemophilus parainfluenzae	33392	3869
Histoplasma capsulatum	11407	3268
Legionella micdadei	33218	2919
Legionella pneumophilia	33152	12323
Mycoplasma pneumoniae (100 ng rRNA)	15531	1884
Nocardia asteroides	19247	3651
Streptococcus pneumoniae	6306	2227

Table 1c: MTD Specificity - Upper Respiratory Tract Organisms

Organism	ATCC Number	RLU Value
Actinomyces pyogenes	19411	2036
Bordetella bronchiseptica	10580	2076
Bordetella pertussis	9797	2425
Candida albicans	18804	2386
Corynebacterium diphtheriae	11913	2685
Corynebacterium haemolyticum	9345	2153
Eikenella corrodens	23834	4130
Enterobacter aerogenes	13048	3345
Enterococcus faecalis	19433	3010
Enterococcus faecium	19434	3061
Klebsiella pneumoniae subsp. ozaenae	11296	3241
Klebsiella pneumoniae	23357	2076
Neisseria meningitidis	13077	2377
Peptostreptococcus magnus	14955	2347
Pseudomonas aeruginosa	25330	2249
Staphylococcus aureus	12598	1983
Staphylococcus epidermidis	12228	1756
Streptococcus pneumoniae	6306	2227
Streptococcus pyogenes	19615	3315

Table 1d: MTD Specificity-Phylogenetic Cross-Sectional Organisms

Organism	ATCC Number	RLU Value
Acinetobacter calcoaceticus	33604	1973
Actinomadura madurae	19425	2866
Actinomyces pyogenes	19411	2036
Actinoplanes italicus	27366	2453
Aeromonas hydrophila	7966	3334
Arthrobacter oxydans	14358	2084
Bacillus subtilis	6051	2570
Brevibacterium linens	9172	2068
Chlamydia trachomatis (50 ng rRNA)	VR878	3189
Chromobacterium violaceum	29094	2306
Citrobacter freundii	8090	3317
Clostridium perfringens	13124	2719
Corynebacterium aquaticum	14665	2304
Corynebacterium genitalium	33030	2792
Corynebacterium matruchotii	33806	2272
Corynebacterium minutissimum	23347	2660
Corynebacterium pseudodiphtheriticum	10700	1855
Corynebacterium pseudogenitalium	33035	2295
Corynebacterium pseudotuberculosis	19410	2362
Corynebacterium renale	19412	3408
Corynebacterium striatum	6940	3525
Deinococcus radiodurans	35073	2412
Dermatophilus congolensis	14637	2212
Derxia gummosa	15994	6646
Erysipelothrix rhusiopathiae	19414	2321
Escherichia coli	10798	2158
Enterobacter cloacae	13047	3248
Fusobacterium nucleatum	25586	2835
Lactobacillus acidophilus	4356	2466
Microbacterium lacticum	8180	2721
Moraxella catarrhalis	25238	2246
Mycoplasma hominis (100 ng rRNA)	14027	1898
Neisseria gonorrhoeae	19424	3718
Nocardia brasiliensis	19296	2529
Nocardia farcinica	3318	2354
Nocardia otitidis-caviarum	14629	3084
Nocardiopsis dassonvillei	23218	2731
Oerskovia turbata	33225	2566

Oerskovia xanthineolytica	27402	2313
Porphyromonas gingivalis	33277	13703
Prevotella melaninogenica	25845	3411
Propionibacterium acnes	6919	2286
Proteus mirabilis	25933	2017
Rahnella aquatilis	33071	2010
Rhodococcus aichiensis	33611	3862
Rhodococcus bronchialis	25592	2932
Rhodococcus chubuensis	33609	2318
Rhodococcus equi	6939	1887
Rhodococcus obuensis	33610	2072
Rhodococcus sputi	29627	14203
Rhodospirillum rubrum	11170	2504
Salmonella enteritidis	13076	3205
Salmonella typhi	6539	2515
Serratia marcescens	13880	3578
Streptococcus bovis	33317	3053
Streptococcus equinus	9812	3504
Streptococcus mitis	9811	2439
Streptococcus sp. Group C	12388	3408
Streptomyces griseus	23345	4691
Veillonella atypica	17744	3185
Vibrio parahaemolyticus	17802	1908
Xanthomonas maltophilia	13637	2885
Yersinia enterocolitica	9610	2066

3. Analytical Sensitivity -- Probe Detection Limits

Thirty strains of *M. tuberculosis* from a wide geographic distribution obtained from CDC, including representative drug-resistant and drug-sensitive strains (Table 2), were tested with the MTD test. All strains were tested in triplicate for 0.05 to 1500 CFU/test (50 μL), diluted in 0.02 percent (v/v) Tween. The MTD test detected 1 to 5 CFU per test for all strains tested (Table 3).

Table 2: *M. tuberculosis* **Strains from the CDC tested in the MTD assay**

Number of Strains	Anti-Tuberculous Drug Sensitivity Profile
10	Drug sensitive
5	INH resistant
1	Rifampin resistant
2	Rifampin and rifabutin resistant
2	Streptomycin resistant
2	INH and rifampin resistant
2	INH, rifampin and rifabutin resistant
2	INH and streptomycin resistant
1	INH and ethambutol resistant
3	INH and ethionamide resistant
30	Total

Table 3: Summary Table - Limits of Detection

Expected CFU/test	$1.5x10^5$ CFU	1500 CFU	500 CFU	50 CFU	5 CFU[a]	0.5 CFU	0.05 CFU
Observed CFU/test (Range)	nd	nd	17-219	1-40	These dilutions correspond to <1 cfu based on observed quantitative culture		
Number dilutions Positive by AFB Smear	30	20	3	Smears not performed on these dilutions.			
Number dilutions Negative by AFB Smear	0	10	27				
MTD Result (Mean RLU)	nd	nd	2,791,018	2,550,354	1,831,705	424,242[a]	147,888(16)[b] 14,544 (14)[c]

[a] 29 of 30 strains were positive in the MTD test at this level

[b] There were 16 observations over the 30,000 RLU cutoff; the mean of these 16 is shown

[c] There were 14 observations under the 30,000 RLU cutoff; the mean of these 14 is shown

nd = not done

4. Precision Studies

Precision panels, consisting of 2 negative samples, 2 low positive samples (83 CFU/test) and 2 moderately high positive samples (750 CFU/test) were tested at Gen-Probe, Inc. and 2 of the sites participating in the clinical studies. The positive samples were prepared by spiking a processed sediment pool with known amounts of *M. tuberculosis*. The samples were tested in triplicate twice a day for 3 days at each of the 3 sites. Positive and negative amplification and hybridization controls were included in each run.

There was no significant site-to-site or day-to-day variability observed. All results agreed with the expected result except for one negative cell control result, which was attributed to a pipetting error. Data from all 3 sites were combined and are shown in Table 4.

Table 4: Precision Studies

	# Observations	% Correct	Range (RLU)	Mean (RLU)
Sample 1 High Positive	108*	100%	76,000 - 3,400,000	3,000,000
Sample 2 Low Positive	108	100%	1,600,000 - 3,300,000	2,900,000
Sample 3 Negative	108	100%	2,700 - 22,000	4,800
Positive Cell Control	54	100%	2,100,000 - 3,300,000	2,900,000
Negative Cell Control	53**	96.2%	2,900 - 29,000	5,500
HPA Positive Control	18	100%	41,000 - 56,000	49,000
HPA Negative Control	18	100%	770 - 2,100	1,100

* One observation - pipetting error
** One observation not performed

5. Test Reproducibility

A reproducibility panel consisting of 25 samples with 1, 10, 25, or 100 cells per test was tested at GenProbe and 3 clinical laboratory sites. Negative controls were interspersed between each sample for a total of 50 samples tested at each site. The following three test sites did not participate in the clinical study:

a. Massachusetts General Hospital;

b. Division of Consolidated Laboratory Services, Commonwealth of Virginia;

c. Public Health Laboratory, County of Orange, CA.

The data are presented in Table 5-a and Table 5-b. For the negative samples, 94.1 percent (113/120) yielded the expected results and 100 percent (80/80) of the positive samples yielded the expected results. One site experienced one, and another site six falsely positive results with negative samples (Table 5-A). After those 2 sites repeated their runs, the overall percentage agreement to expected results was 97.5 percent (117/120, with three false positive results at one site and none at the other) for the negative samples and 100 percent (80/80) for the positive samples (Table 5).

The falsely positive results were attributed to inadequate water bath levels and/or insufficient vortexing in the procedure. Precautions for these procedural aspects have been included in the device labeling. The reproducibility of the assay will be further assessed in post-approval studies.

Table 5-a Reproducibility Testing

Sample #	Cell Level	Site 1	Site 2	Site 3	Site 4
1	Negative	8035	2240	8028	6359
2	1 cell	1806647	344395	310930	357667
3	Negative	58719	2344	49386	5247
4	10 cells	>2,000,000	402710	786523	>2,000,000
5	Negative	6815	2247	7461	5590
6	25 cells	>2,000,000	1842613	>2,000,000	>2,000,000
7	Negative	9509	2820	7588	4832
8	100 cells	>2,000,000	>2,000,000	>2,000,000	>2,000,000
9	Negative	7856	2090	9821	7845
10	1 cell	1317704	290186	827208	232822
11	Negative	16366	1837	3995	6243
12	Negative	8624	2375	55534	5239
13	Negative	7950	2112	13402	7419
14	50 cells	>2,000,000	>2,000,000	>2,000,000	>2,000,000
15	Negative	8445	1981	18363	5405
16	Negative	7365	1953	9146	5460
17	Negative	8724	1813	3806	7411
18	10 cells	>2,000,000	997875	>2,000,000	>2,000,000
19	Negative	8066	1904	24326	8769
20	50 cells	>2,000,000	1879054	>2,000,000	>2,000,000
21	Negative	7111	1990	48043	5370
22	25 cells	>2,000,000	>2,000,000	>2,000,000	>2,000,000
23	Negative	8619	2135	25836	4419
24	100 cells	>2,000,000	>2,000,000	>2,000,000	>2,000,000
25	Negative	7364	2035	12294	6185
26	Negative	9520	2030	12572	6066
27	1 cell	>2,000,000	137038	204783	278503
28	Negative	8746	1880	4595	4861
29	10 cells	>2,000,000	782635	1226740	1245660
30	Negative	8641	1970	4058	6816
31	25 cells	>2,000,000	1527896	1981069	>2,000,000
32	Negative	8355	1875	4718	5279
33	100 cells	>2,000,000	1692113	>2,000,000	>2,000,000
34	Negative	8303	1940	10535	4915
35	1 cell	1641990	140678	1172246	141800
36	Negative	8416	1874	8741	5837
37	Negative	9693	1904	7183	5148
38	Negative	7990	1695	4768	5198

39	50 cells	>2,000,000	>2,000,000	>2,000,000	>2,000,000
40	Negative	8035	1663	6004	5383
41	Negative	8343	1876	6445	6868
42	Negative	8791	6016	59309	6086
43	10 cells	>2,000,000	838212	1827546	1712028
44	Negative	9643	1770	34428	4984
45	50 cells	>2,000,000	931028	>2,000,000	>2,000,000
46	Negative	8529	1777	5410	5539
47	25 cells	>2,000,000	777363	>2,000,000	>2,000,000
48	Negative	8209	1793	124645	7050
49	100 cells	>2,000,000	1554216	>2,000,000	>2,000,000
50	Negative	10047	2054	4944	7426
Pos Cell Ctrl		>2,000,000	>2,000,000	>2,000,000	>2,000,000
Neg Cell Ctrl		8306	1980	2730	5955

Site 1: Orange County
Site 2: Mass General
Site 3: Commonwealth of Virginia
Site 4: Gen-Probe

Table 5-b Repeat Reproducibility Testing

Sample #	Cell Level	Site 1	Site 3
1	Negative	10470	172247
2	1 cell	>2,000,000	1431068
3	Negative	13148	4351
4	10 cells	>2,000,000	>2,000,000
5	Negative	8046	106879
6	25 cells	>2,000,000	>2,000,000
7	Negative	9101	3328
8	100 cells	>2,000,000	>2,000,000
9	Negative	8862	4311
10	1 cell	854525	>2,000,000
11	Negative	11844	3885
12	Negative	8887	3480
13	Negative	7566	3179
14	50 cells	>2,000,000	>2,000,000
15	Negative	7220	3014
16	Negative	10422	3474
17	Negative	9067	2951
18	10 cells	>2,000,000	>2,000,000

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19	Negative	8899	1034320
20	50 cells	>2,000,000	>2,000,000
21	Negative	9866	3423
22	25 cells	>2,000,000	>2,000,000
23	Negative	6614	3166
24	100 cells	>2,000,000	>2,000,000
25	Negative	7338	3490
26	Negative	8337	3536
27	1 cell	1378301	834358
28	Negative	8668	3453
29	10 cells	>2,000,000	>2,000,000
30	Negative	7139	3346
31	25 cells	>2,000,000	>2,000,000
32	Negative	7401	4350
33	100 cells	>2,000,000	>2,000,000
34	Negative	7260	3089
35	1 cell	1320964	1983828
36	Negative	7393	3773
37	Negative	7539	5965
38	Negative	9045	3186
39	50 cells	>2,000,000	>2,000,000
40	Negative	10323	4150
41	Negative	14775	3758
42	Negative	9474	3368
43	10 cells	>2,000,000	>2,000,000
44	Negative	8745	3062
45	50 cells	>2,000,000	>2,000,000
46	Negative	9132	3441
47	25 cells	>2,000,000	>2,000,000
48	Negative	12612	4442
49	100 cells	>2,000,000	>2,000,000
50	Negative	6637	3763
Pos Cell Ctrl		>2,000,000	>2,000,000
Neg Cell Ctrl		9009	3800

Site 1: Orange County

Site 3: Commonwealth of Virginia

6. Interference Studies

In 4 separate studies, testing was performed to determine if any of the following agents interfered

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with detection of *M. tuberculosis* with the MTD test: blood, nebulizing solution, human leukocyte DNA, and non-target organisms.

The presence of 2.5 percent (v/v) blood in sputum that was digested and decontaminated did not interfere with the amplification of 0, 5, 100, or 300 CFU/test of *M. tuberculosis*. Five percent (5 percent) and 10 percent (v/v) blood in sputum did not inhibit amplification of 100 and 300 CFU/test, but the 5 CFU sample was falsely negative in the presence of 5 and 10 percent blood with the MTD (Table 6). This data demonstrated that amounts of blood greater than 2.5 percent would interfere with MTD results. Warnings and limitations to the use of the device with specimens that are bloody have been included in the labeling.

Table 6: Effect of Blood on the MTD Test

CFU	% Blood	Sample 1 (RLU)	Sample 2 (RLU)	Sample 3 (RLU)	Sample 4 (RLU)
0	0	2126	2784	2616	2998
0	2.5%	2213	2364	2785	2478
0	5%	2569	3045	3089	3014
0	10%	2815	3797	3572	3291
5	0	2742444	2725540	2829926	2827946
5	2.5%	1018835	2756836	3081203	2768209
5	5%	12792	2459608	2855343	2666631
5	10%	1492569	17826	147481	331902
100	0	2647256	2732357	2855961	2949178
100	2.5%	2569621	2715612	2989072	2735458
100	5%	2505845	2652691	2906096	2747376
100	10%	1447482	2593406	206484	2911446
300	0	2701849	2701145	2998767	2852130
300	2.5%	2678630	2690448	3018682	2775899
300	5%	2586551	2676773	3062163	2829110
300	10%	2520516	2617977	2921338	2727120

Normal saline, the only nebulizing solution used in the trial, was selected for evaluation at concentrations of 1.5 percent and 3 percent. There

was no effect on either positive or negative samples from either concentration of saline on the MTD test results for either positive or negative samples.

Because respiratory specimens are known to contain human nucleic acid, MTD test performance was assessed in the presence of varying amounts of human white blood cell DNA (WBC-DNA) per test. No interference with the *M. tuberculosis* signal was observed at concentrations of *M. tuberculosis* ranging from 2.5 fg (equivalent to one-half cell) to 250 fg (equivalent to 50 cells) in the presence of 100 ng (0.0001 pg) and 0.085 mg WBC-DNA. In the presence of 0.017 and 0.85 mg human WBC-DNA, 5 fg *M. tuberculosis* rRNA (equivalent to one cell) was not detected. A total of 50 fg *M. tuberculosis* rRNA was detected in all levels of WBC-DNA tested. The MTD results for negative specimens were not affected by the presence of any amount of WBC-DNA. This data demonstrated that extreme purulence in specimens (such as in highly mucopurulent specimens from cystic fibrosis patients that may contain up to 15 mg of DNA/mL) could interfere with the MTD results.

To determine whether non-target organisms would interfere with the MTD test, *M. tuberculosis* rRNA (5 fg) was tested in the presence and absence of 12 species of bacteria. The species were either closely related to *M. tuberculosis* or species known to cause respiratory disease. All positive test results remained positive in the presence of 290,000 CFU/test of non-target organisms. The results are shown in Table 7. RLU levels were reduced in the presence of *P. aeruginosa, M. gordonae, M. avium, G. sputi, Nocardia otitidis-caviarum, and R. broncialis*. Since higher levels of organisms were not tested, it was inferred that large numbers of MOTT and closely related species that are amplified but do not hybridize with the Detector Probe in the MTD, may have reduced RLU signal.

Table 7: Amplification of 5 fg of *M. tuberculosis* rRNA in the presence or absence of other bacteria

		RLU*		
Bacterial Species	ATCC No.	290,000 CFU per reaction	2,900 CFU per reaction	0 CFU per reaction
Haemophilus influenzae	19418	1,352,456	2,149,025	2,001,148
Streptococcus pneumoniae	33400	1,814,873	2,119,640	2,065,117
Legionella pneumophila	33152	899,639	2,206,019	2,003,331
Pseudomonas aeruginosa	9027	213,624	1,865,116	2,073,948
Mycobacterium gordonae	14470	546,980	2,102,254	2,154,300
Mycobacterium avium	25291	697,861	1,901,832	2,070,336
Nocardia asteroides	19427	3,029,602	2,875,657	2,699,944
Corynebacterium pseudotuberculosis	19410	2,978,896	2,686,641	2,683,828
Corynebacterium diphtheriae	e11913	2,930,603	2,780,569	2,687,980
Gordona sputi	29267	152,273	2,843,971	2,804,503
Nocardia otitidis-caviarum	14629	469,055	2,952,836	2,747,145
Rhodococcus bronchialis	25592	286,335	2,840,834	2,743,153

*cutoff 30,000 RLU; all results are mean of triplicates

Inhibition effects were assessed by testing MTD results that were discordant with culture results. Of the seven smear positive specimens in the clinical study that were MTD negative and culture positive, 6 were tested retrospectively from frozen samples with an rRNA spike-in procedure. Two of the 6 samples tested showed inhibition, defined as a negative MTD result when rRNA was added to the frozen specimen. Additional post-approval studies will assess the effectiveness of using this type of procedure to monitor for specimen inhibition effects on MTD results.

B. Clinical Studies

The objectives of this prospective multicenter clinical trial were to assess the performance of the MTD test for detection of *M. tuberculosis* complex rRNA in AFB smear positive respiratory specimens compared to standard laboratory culture, and to assess the clinical utility of this test as an adjunctive test to mycobacterial culture.

The MTD test was evaluated with specimens collected from patients at the 6 geographically diverse sites shown below. The sites included 5 large metropolitan hospital centers and one state public health laboratory:

1. SUNY Health Science Center, Brooklyn, New York (BRK);

2. Illinois Department of Public Health, Chicago, Illinois (CHI);

3. Columbia-Presbyterian Medical Center, New York, New York (COL);

4. Veterans Administration Medical Center, Houston, Texas (HOU);

5. Jackson Memorial Hospital, Miami, Florida (MIA);

6. UCSD Medical Center, San Diego, California (UCSD).

The study population represented all patients from whom lower respiratory tract specimens were submitted for AFB smear and culture during the study period (6,079 specimens from 2,609 patients). These included patients not on anti-tuberculous therapy or not having received such therapy for 7 days or more(4,000 specimens from 1,898 patients), patients currently on such therapy and patients with a history of such therapy. Of those patients considered not on therapy at the time of specimen collection, 198 specimens from 78 patients were AFB-smear positive.

The specimens were evaluated by AFB smear, culture, and the MTD test. The sites used their established protocols, which were adaptations of CDC's guidelines for NALC-NAOH digestion and decontamination of specimens for AFB smear and culture. Other methods of digestion and decontamination were not evaluated. All AFB smears were performed using a fluorochrome stain (auramine with or without rhodamine). AFB smears were graded by the relative numbers of AFB seen on the smear according to ATS/CDC recommendations.[1,6] Mycobacterial growth from cultures were identified as *M. tuberculosis* complex by hybridization with specific probes and/or biochemical characterization. Culture was considered *M. tuberculosis* complex positive for any one patient if at least 1 culture for any specimen was positive. All sites used radiometric liquid media with at least one solid media except for one site that used 2 different solid media only.

The MTD test was compared to the composite results of standard culture methods routinely used at each site.

For those specimens with MTD positive results and for which the companion culture failed to grow *M. tuberculosis*, the MTD result was considered a true positive if other specimens from the same patient collected within the same time frame were culture positive for *M. tuberculosis*. Of the 198 AFB smear positive specimens (from 78 patients not on therapy) tested with the MTD, 145 were culture positive for *M. tuberculosis* complex; 2 of these were further identified as *M. bovis*. MTD detected 138 of these culture positive specimens; 1 specimen also grew MOTT in addition to *M. tuberculosis*. Nine additional specimens were MTD positive when the companion culture was negative; however, for these patients, other cultures were positive for *M. tuberculosis*.

Table 8: MTD vs. Culture in Smear Positive Samples from Untreated Patients (N=198)

		Culture +	Culture -
MTD	+	147	0
	-	7	44

The overall MTD test sensitivity was 95.5 percent (147/154) and specificity was 100 percent (44/44) compared to culture results. The ranges at the 6 sites for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are also shown in Table 9, along with the 95 percent confidence intervals for the performance estimates. Results by site are shown in Table 10.

Table 9: Sensitivity, Specificity, and Predictive Values

	Overall Percentage	Number versus Total	Range of Percentages at Six Sites	95% Confidence Interval
Sensitivity	95.5%	147/154	85.7-100%	90.9-98.2%
Specificity	100%	44/44	100%	93.4-100%
PPV	100%	147/147	100%	98.0-100%
NPV	86.3%	44/51	76.0-100%	73.7-94.3%

Table 10: Results by site

Site	Sensitivity %(TP/FN + TP)	Specificity %(TN/TN + FP)
BRK	100 (67/67)	100 (3/3)
CHI	100 (2/2)	N.D. (0/0)
COL	100 (4/4)	100 (6/6)
HOU	96.0 (24/25)	100 (14/14)
MIA	85.7 (36/42)	100 (19/19)
UCSD	100 (14/14)	100 (2/2)
TOTAL	95.5 (147/154)	100 (44/44)

N.D. = not determined

Of the 7 specimens that were negative with the MTD test but positive for *M. tuberculosis* complex by culture, 2 were from specimens with MOTT/*M. tuberculosis* mixed cultures. Six (6) of the 7 specimens were tested for inhibition using a spike-in procedure with *M. tuberculosis* RNA in frozen reserved sediment; 2 specimens inhibited detection of the RNA.

Of the 44 AFB smear positive specimens that were culture and MTD negative for Mtb complex: 33 specimens grew MOTT on culture, 4 specimens were from patients with other cultures positive for MOTT, 3 specimens were from a single patient with negative mycobacterial cultures but clinically diagnosed reactivated TB, and 4 specimens were from patients with negative mycobacterial cultures and who were not clinically diagnosed with TB.

VIII. Conclusions Drawn from the Studies

The analytical and clinical study data analyses demonstrated that the the MTD could detect *M. tuberculosis* complex rRNA in AFB smear positive respiratory specimens collected from untreated patients, using the RLU cutoff determined analytically.

In analytical studies, the MTD test detected 30 strains of *M. tuberculosis* isolated from patients in different geographical areas, including drug-resistant and drug-sensitive strains at a level of detection of 1-5 CFU per

test. *M. celatum* and *M. terrae-like* strains also hybridize with the MTD Detector Probe; these species, however are rarely encountered as clinical isolates. Other pathogenic and normal flora species tested did not cause positive results.

Precision testing demonstrated that with repetitive testing of selected samples, the MTD produced expected results. A reproducibility study performed in the manner of a proficiency assessment revealed technical factors (i.e., water bath levels, pipetting, vortexing procedures, potential amplicon contamination) that may result in false positive results with the MTD.

In interference studies, grossly bloody specimens and high levels of WBC-DNA were shown to interfere (falsely negative results in samples with *M. tuberculosis* cells or rRNA present) with the MTD test. Non-target organisms (e.g., MOTT) that were also amplified in the MTD reduced the RLU signal when present in high numbers. At the levels of organisms tested, however, no samples with *M. tuberculosis* rRNA present had false negative results.

Due to confounding factors with the clinical assessment of patients, MTD performance was assessed in comparison to AFB culture results. This analysis showed a high degree of specificity compared to culture for smear positive specimens. The reduced sensitivity of the MTD relative to culture may be adjusted if those specimens that have inhibitors present can be identified. Inhibition and other specimen variables (e.g., method of digestion and decontamination used) may cause false negative results.

CDRH has concluded that the device is safe and effective for the stated intended use when the device procedure is performed with strict adherence to the directions for use and when additional controls are in place to control for inhibition effects

IX. Panel Recommendation

On May 2, 1995, the FDA Microbiology Devices Panel voted to approve the MTD test with conditions. The conditions specified by the Panel were not absolute, and final form was acknowledged to be subject to negotiations between the FDA and the Sponsor. The conditions were:

A. Use of the MTD is to be limited to AFB smear positive sediments from patients not on therapy. Data to support indications for use with AFB smear negative

sediments could be considered if the applicant resubmits the data in a form that supports such an indication.

B. Incorporation of a control for specimen inhibition into the assay procedure.

C. Providing directions for using a contamination monitoring procedure.

D. Clarification in the labeling of methods to use recommended controls to monitor device performance.

E. Restricting the use of the test to microbiology laboratories maintaining proficiency in mycobacterial culture and identification of *M. tuberculosis*.

F. Performing post-approval studies to provide additional information for the following effects on MTD detection of *M. tuberculosis* rRNA:

- freezing specimens
- excess NaOH exposure and use of different sediment resuspension fluids during digestion and decontamination procedures
- extended storage of specimens on MTD detection of *M. tuberculosis* rRNA, and
- effects of buffer used to re-suspend sediments.

X. FDA Decision

CDRH concurred with the recommendation of the Panel. CDRH issued an approvable letter to the applicant on August 28, 1995, requesting post-approval studies to monitor the recommended specimen inhibition control, contamination monitoring procedure, and reproducibility of the device using fresh and frozen samples. An approval order was issued for the applicant's PMA for Gen-Probe® Amplified Mycobacterium Tuberculosis Direct Test to Gen-Probe, Incorporated on December 15, 1995.

The applicant's manufacturing and control facilities were inspected on April 13, 1995, and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs). The shelf-life of the Gen-Probe® Mycobacterium Tuberculosis Direct Test has been established at 12 months stored at 2-8°C.

XI. Approval Specifications

Directions for Use: See attached labeling

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Conditions of Approval: CDRH approval of the PMA is subject to compliance with the conditions described in the approval order

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GEN-PROBE®

AMPLIFIED

AMPLIFIED MYCOBACTERIUM TUBERCULOSIS DIRECT TEST

For *In-vitro* Diagnostic Use

50 Test Kit

INTENDED USE

The Gen-Probe Amplified Mycobacterium Tuberculosis Direct (MTD) Test is a target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid fast bacilli (AFB) smear positive concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates) or tracheal aspirates.

The MTD test is to be used as an adjunctive test for evaluating AFB smear positive sediments prepared using NALC-NaOH digestion-decontamination of respiratory specimens from untreated patients suspected of having tuberculosis. Patients who have received no antituberculous therapy, less than 7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated with this test. The MTD test should be performed only in laboratories proficient in the culture and identification of *M. tuberculosis* (Level II and III or extent 3 and 4) [1]. The MTD should always be performed in conjunction with mycobacterial culture.

WARNINGS

The efficacy of this test has not been demonstrated for the direct detection of *M. tuberculosis* rRNA using other clinical specimens (e.g., blood, urine, or stool). Performance of the MTD test has not been established for sediments processed in a different fashion than described, or stored for different time periods or temperatures than specified in this Package Insert.

Positive sediments must be cultured to determine if Mycobacteria other than *M. tuberculosis* (MOTT) are present in addition to *M. tuberculosis* complex and to perform antimycobacterial susceptibility testing. Culture for AFB should also be performed to determine which subspecies of the *M. tuberculosis* complex (e.g. *M. bovis*) is present.

AFB smear positive samples may be MTD test negative and *M. tuberculosis* complex culture positive. This condition may be caused by inhibition of the MTD test or the presence of low levels of the *M. tuberculosis* complex organism in the presence of large numbers of MOTT.

Although specimens from pediatric patients, HIV positive patients and patients with MOTT infections were tested during the clinical evaluations, total numbers were insufficient to definitely conclude that there were no statistical performance differences in these specific patient populations.

The MTD test has not been studied for use with specimens from patients being treated with antituberculous agents to determine bacteriologic cure or to monitor response to such therapy.

Specimens that are grossly bloody should not be tested with the MTD.

PRECAUTIONS

A For *In Vitro* Diagnostic Use.

B The MTD test is specific for, but does not differentiate among, members of the *M. tuberculosis* complex, i.e., *M. tuberculosis, M. bovis, M. bovis* BCG, *M. africanum*, and *M. microti*. *M. celatum* and *M. terrae*-like organisms will cross react if present at concentrations higher than 30 CFU per test. However, *M. celatum* or *M. terrae*-like organisms are rare clinical isolates.

C A negative test does not exclude the possibility of isolating an *M. tuberculosis* complex organism from the specimen. Test results may be affected by specimen collection and transport, specimen sampling variability, laboratory procedural errors, sample misidentification, and transcriptional errors.

D. Use only for the detection of members of the *M. tuberculosis* complex using sediments prepared following the NALC/NaOH and NaOH procedures recommended by the CDC[7]. This test may only be used with concentrated sediments prepared from sputum (induced or expectorated), tracheal aspirates, or bronchial specimens (e.g., bronchoalveolar lavages and bronchial aspirates).

E. Avoid contact of Detection Reagents I and II with skin, eyes and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur dilute with water before wiping dry.

F. Use universal precautions when performing this test[4]. Preparation of digested and decontaminated concentrates, and MTD procedures should be done using Biosafety Level 2 practices[5].

G. Use only supplied or specified disposable laboratory ware.

H. Pipetting steps as described in Test Procedure should be performed in a laminar flow biological safety cabinet.

I. Work surfaces, pipettors, and equipment must be decontaminated with a 1:1 dilution of household bleach as described in the Test Procedure. Work surface may be wiped with water to remove the bleach.

J. Positive displacement pipettors or air displacement pipettors with hydrophobically plugged tips must be used when performing this test. When transferring lysate from Lysing Tube to Amplification Tube, extended length hydrophobically plugged tips must be used. A separate disposable tip must be used for each reaction tube. Waving of a pipette tip containing specimen over the rack of tubes should be avoided. Spent pipette tips must be immediately discarded in an appropriate biosafety waste container.

K. When using repeat pipettors for reagent addition, after the lysate has been added to the tube, avoid touching the tube with the pipette tip in order to minimize the chance of carryover from one tube to another. The reagent stream should be aimed against the interior wall of the test tube to prevent splashing. Careful pipetting is important to avoid carryover contamination.

L. After reading reaction tubes in the luminometer, decontaminate and carefully dispose of them as described in the Test Procedure and Procedural Notes in order to avoid contamination of the laboratory environment with amplicon.

M. Sealing cards or snap caps should be disposed of in an appropriate biosafety waste container immediately after removing them from reaction tubes. Fresh sealing cards or snap caps should always be used to avoid cross contamination. These materials should NEVER be reused from a previous step. Sealing cards should be firmly fixed to the top of all reaction tubes.

N. Do not cover water bath during incubations, especially when using snap caps. (Condensation from the cover may be a possible source of contamination.)

SUMMARY AND EXPLANATION OF THE TEST

The MTD test utilizes Transcription Mediated Amplification (TMA) and the Hybridization Protection Assay (HPA) to qualitatively detect *M. tuberculosis* complex ribosomal ribonucleic acid (rRNA). The MTD test will detect rRNA from both cultivable and non-cultivable organisms. Organisms of the *M. tuberculosis* complex include *M. tuberculosis, M. bovis, M. bovis* BCG, *M. africanum*, and *M. microti*[12]. The MTD test will detect all organisms within the *M. tuberculosis* complex. However, *M. microti* infects only animals, *M. bovis* is uncommonly transmitted from infected animals to humans, and *M. africanum* causes pulmonary disease in humans in tropical Africa[12]. *M. tuberculosis* is by far the most common member of the complex that is responsible for human disease worldwide. The Centers for Disease Control and Prevention (CDC) has recently reported a rise in the incidence of tuberculosis associated with AIDS, foreign-born cases, and increased transmission in higher risk populations[8,9]. There has also been a rise in the number of *M. tuberculosis* strains that are resistant to one or more than one anti-tuberculous drugs[11]. The public health implications of these facts are considerable.

Conventional culture methodologies can detect tuberculosis growth as early as 1 week, but may take up to 8 weeks[7,10]. Comparatively, the MTD test provides detection of *M. tuberculosis* complex rRNA within 4 to 5 hours after beginning the test procedure. Thus, while the MTD test cannot ascertain drug susceptibility, it can result in rapid and reliable detection of *M. tuberculosis*. This could lead to more appropriate use of isolation facilities, more appropriate initiation of therapy, and earlier detection and containment of infected contacts[3].

PRINCIPLES OF THE PROCEDURE

The MTD test is a two-part test in which amplification and detection take place in a single tube. Initially, nucleic acids are released from mycobacterial cells by sonication. Heat is used to denature the nucleic acids and disrupt the secondary structure of the rRNA. The Gen-Probe Transcription Mediated Amplification method, using a constant 42°C temperature, then amplifies a specific mycobacterial ribosomal RNA target by transcription of DNA intermediates, resulting in multiple copies of the mycobacterial RNA (amplicon).

M. tuberculosis complex-specific sequences are then detected in the amplified ribosomal RNA (amplicon) using the Gen-Probe Hybridization Protection Assay method[2]. The Mycobacterium Tuberculosis Probe Reagent is a chemiluminescent-labeled, single-stranded DNA probe that is complementary to *M. tuberculosis* complex specific sequences. Once stable RNA:DNA hybrids are formed between the probe and the specific sequences, hybridized probe is selected and measured in a Gen-Probe LEADER® luminometer.

REAGENTS (50 Test Kit)

Reagents for the MTD test are provided as follows:

Reagent Name	Volume
Mycobacterium Lysing Tubes *Glass Beads, Bulking Agent*	2 x 25 Tubes
Mycobacterium Specimen Dilution Buffer (1) *Tris buffered solution containing ≥ 5mM N-acetyl-L-cysteine*	1 x 10 mL
Mycobacterium Tuberculosis Amplification Reagent (2A) *Nucleic acids lyophilized in Tris buffered solution containing additives.*	2 x 0.75 mL when reconstituted
Mycobacterium Reconstitution Buffer (2B) *Aqueous solution containing preservatives.*	1 x 1.5 mL
Mycobacterium Oil Reagent (3) *Silicone Oil*	1 x 10 mL
Mycobacterium Enzyme Reagent (4A) *Reverse transcriptase and RNA polymerase lyophilized in HEPES buffered solution containing <10% bulking agent.*	1 x 1.4 mL when reconstituted
Mycobacterium Enzyme Dilution Buffer (4B) *Tris buffered solution containing surfactant, glycerol, and 1.5 mM N-acetyl-L-cysteine.*	1 x 1.5 mL
Mycobacterium Termination Reagent (5) *Enzyme in buffered solution containing glycerol.*	1 x 1 mL
Mycobacterium Tuberculosis Hybridization Positive Control (HP) *≥ 500 pg/mL non-infectious nucleic acid.*	1 x 1 mL
Mycobacterium Hybridization Negative Control (HN) *Phosphate buffered solution.*	1 x 1 mL
Mycobacterium Tuberculosis Probe Reagent (6A) *< 5 pg/vial chemiluminescent labeled non-infectious DNA lyophilized in succinate buffered solution containing bulking agent and detergent.*	1 x 6 mL when reconstituted
Mycobacterium Hybridization Buffer (6B) *Buffered solution*	1 x 6 mL
Mycobacterium Selection Reagent (7) *Borate buffered solution containing surfactant*	1 x 15 mL

STORAGE AND HANDLING REQUIREMENTS

A. The following liquid or unreconstituted components must be stored between 2-8°C and are stable until the expiration date indicated on the vial label:

Mycobacterium Specimen Dilution Buffer (1)
Mycobacterium Tuberculosis Amplification Reagent (2A)
Mycobacterium Reconstitution Buffer (2B)
Mycobacterium Enzyme Reagent (4A)
Mycobacterium Enzyme Dilution Buffer (4B)
Mycobacterium Tuberculosis Probe Reagent (6A)
Mycobacterium Termination Reagent (5)

The reconstituted Mycobacterium Amplification Reagent (2A) is stable for 2 months at 2°-8°C. The Mycobacterium Tuberculosis Probe Reagent (6A) and the Mycobacterium Enzyme Reagent (4A) are stable at 2°-8°C for 1 month after reconstitution.

B. The following kit components are stable when stored at 2°-25°C until the expiration date indicated on the vial label.

Mycobacterium Oil Reagent (3)
Mycobacterium Tuberculosis Hybridization Positive Control (HP)
Mycobacterium Hybridization Negative Control (HN)
Mycobacterium Hybridization Buffer (6B)
Mycobacterium Selection Reagent (7)
Mycobacterium Lysing Tubes

SPECIMEN COLLECTION, STORAGE, TRANSPORT, AND PROCESSING

Specimen Collection and Storage:
Specimens must be collected in sterile, plastic containers, and stored at 2°-8° C until transported or processed. Specimens should be processed (decontaminated and concentrated) within 24 hours of collection (including transport time) as recommended by the CDC. Specimens tested in the clinical trial were tested within 72 hours (generally within 24 hours).

Transport:
Transport freshly collected or refrigerated specimens to the laboratory as soon as possible. The shipment outside the collection facility of diagnostic specimens such as sputum, etc., and cultures including all *Mycobacterium ssp.* is subject to the packaging and labeling requirements of the Interstate Quarantine Regulation (Federal Register, Title 42, Chapter 1, Part 72, Revised July 30, 1972)[7]

Processing (Decontamination and Concentration):
Specimens that are grossly bloody should not be tested with the MTD test. The MTD test is designed to detect rRNA from members of the *M. tuberculosis* complex using sediments prepared from generally accepted current adaptations of the NALC/NaOH or NaOH decontamination protocols described by the CDC using 1% to 1.5% NaOH for 15-20 minutes and centrifugation at ≥ 3,000 x *g*.[7]

Processed Sediment Storage:
Sediments may be stored at 2°-8°C for up to 3 days prior to testing.

MATERIALS

Materials Provided

Cat. No. 1001	50 tests
Mycobacterium Specimen Dilution Buffer (1)	1 X 10 mL
Mycobacterium Tuberculosis Amplification Reagent (2A)	2 x 0.75 mL
Mycobacterium Reconstitution Buffer (2B)	1 x 1.5 mL
Mycobacterium Lysing Tubes	2 x 25 Tubes
Mycobacterium Oil Reagent (3)	1 x 10 mL
Mycobacterium Enzyme Reagent (4A)	1 x 1.4 mL (when reconstituted)

Mycobacterium Enzyme Dilution Buffer (4B)	1 x 1.5 mL
Mycobacterium Termination Reagent (5)	1 x 1 mL
Mycobacterium Tuberculosis Hybridization Positive Control (HP)	1 x 1 mL
Mycobacterium Hybridization Negative Control (HN)	1 x 1 mL
Mycobacterium Tuberculosis Probe Reagent (6A)	1 x 6 mL (when reconstituted)
Mycobacterium Hybridization Buffer (6B)	1 x 6 mL
Mycobacterium Selection Reagent (7)	1 x 15 mL
Sealing Cards	1 package

Materials Required But Not Provided

Water bath and/or dry heat bath* (42° ± 1°C and 60° ± 1°C) and dry heat bath* (95° ± 5°C)
Micropipettes capable of dispensing 20 µL, 25 µL, 50 µL, 100 µL, 200 µL, and 300 µL
Vortex mixer
Sterile water (filtered or autoclaved)
Culture tubes
3 mm glass beads
Screw cap microcentrifuge tubes
Positive Cell Controls (e.g., *M. tuberculosis*, ATCC 25177 or ATCC 27294)
Negative Cell Controls (e.g., *M. gordonae*, ATCC 14470, or *M. terrae*, ATCC 15755)
Household Bleach (5.25% hypochlorite solution)
Plastic Backed Bench Covers

Additional Materials Required and Available From Gen-Probe:

Gen-Probe LEADER Luminometer
Gen-Probe Sonicator
Gen-Probe Detection Reagent Kit
Gen-Probe Dry Heat Bath
Gen-Probe Sonicator Rack
Pipette tips with hydrophobic plugs
Tubes, polypropylene, 12 x 75 mm
Micropipettes capable of dispensing 20 µL, 25 µL, 50 µL, 100 µL, 200 µL, and 300 µL
Snap top polypropylene caps for 12 x 75 mm tubes

* Heating blocks must have wells properly sized for 12 x 75 mm tubes. Use of Gen-Probe dry heat bath is recommended.

TEST PROCEDURE

Equipment Preparation

1. For optimal transfer of sonic energy in sonicator, water must be thoroughly degassed according to the following procedure prior to each run:
   a. Add enough ambient temperature tap water to fill the sonicator bath to within 1/2 inch of the top of the tank.
   b. Run the sonicator for 15 minutes to thoroughly degas the water.
2. Adjust 1 dry heat bath to 95° C, 1 dry heat bath or water bath to 60° C and another dry heat bath or water bath to 42° ± 1°C.
3. Wipe down work surfaces, equipment, and pipettors with a 1:1 dilution of household bleach prior to starting. Bleach must be in contact with the surface for at least 15 minutes. Work surfaces may be wiped with water to remove the bleach. Cover the surface on which the test will be performed with plastic-backed laboratory bench covers.
4. Prepare the Gen-Probe LEADER Luminometer for operation. Make sure there are sufficient volumes of Detection Reagents I and II to complete the tests.

Reagent Preparation

Reconstitute 1 vial (25 tests) of lyophilized Mycobacterium Tuberculosis Amplification Reagent (2A) with 750 µL Mycobacterium Reconstitution Buffer (2B). Vortex until the solution is mixed. Let reconstituted reagent sit at room temperature until clear. The *reconstituted* Mycobacterium Tuberculosis Amplification Reagent may be stored at 2°-8°C for 2 months. The *reconstituted* Mycobacterium Tuberculosis Amplification Reagent should be allowed to come to room temperature before use.

Controls

1. Lysis and Amplification Controls

   Cells used for the Cell Positive Control should be a member of the *M. tuberculosis* complex, such as, *M. tuberculosis*, (ATCC #25177 or 27294) suspended in sterile water. Cells used for the Cell Negative Control should be mycobacteria other than tuberculosis complex, such as *M. gordonae* (ATCC #14470) or *M. terrae* (ATCC #15755).

   Preparation and Storage of Amplification Controls

   a. Place 3 to 5 sterile 3 mm glass beads in a clean culture tube.
   b. Add 1-2 mL sterile water. Add several 1 µL loopfuls of growth from the appropriate culture. Cap the tube and vortex repeatedly at high speed.
   c. Allow the suspension to settle for 15 minutes.
   d. Transfer the supernatant to a clean culture tube. Adjust turbidity to the equivalent of a #1 McFarland nephelometer standard using a McFarland reference.
   e. Make a 1:100 dilution (Dilution 1) of the suspension by placing 100 µL of the #1 McFarland suspension into 10 mL sterile water. Cap and vortex.
   f. Make a second 1:100 dilution (Dilution 2) by placing 100 µL Dilution 1 into 10 mL sterile water. Cap and vortex.
   g. The final dilution is then made by taking 100 µL of Dilution 2 and placing it into 6 mL of sterile water. Cap and vortex. The final dilution (Dilution 3) contains approximately 25 CFU per 50 µL.
   h. Plate 50 µL of each dilution onto culture medium and freeze remaining stock at -20°C or -70°C. Test all dilutions using the MTD test.
   i. The dilutions that give between 25 and 150 CFU per 50 µL on the plated culture medium, and that perform as expected with the MTD test should be thawed, aliquoted, and used as controls.
   j. The dilutions must be aliquoted into clean 1.5 mL screw cap microcentrifuge tubes as single use aliquots (100 µL) and stored frozen at -20°C or -70°C. The tubes may be stored at -20°C for 6 months or -70°C for 1 year. Frost-free freezers must not be used.

   A single replicate of the cell controls must be tested with each run.

   Each laboratory should determine target values and means for the controls. See "Test Interpretation- A. Quality Control Results and Acceptability" section of this Package Insert.

2. Hybridization Controls

   Mycobacterium Tuberculosis Hybridization Positive Controls (HP) and Mycobacterium Hybridization Negative Control (HN) are provided in the MTD test kit. A single replicate of each of the Hybridization Controls should be tested with each run for quality control purposes.

3. Specimen Inhibition Controls

   When the AFB smear is positive and the MTD test is negative for untreated patient specimens, there are 3 conditions that might exist:

(1) the specimen is inhibitory, or
(2) the specimen contains *Mycobacterium* other than tuberculosis complex (MOTT), or
(3) the specimen contains a mixture of large numbers of MOTT and a low number of *Mycobacterium tuberculosis* complex organisms.

To test for specimen inhibition, the following procedure should be performed:

Protocol - Testing Patient Sediments for Inhibition in the MTD Test.

a. Place 200 µL Specimen Dilution Buffer into 2 Lysing Tubes (seeded and unseeded).
b. Add 50 µL Amplification Positive Cell Control and 50 µl sediment to 1 tube (seeded). Add 50µL sediment to the second tube (unseeded). Proceed with the testing as usual.

Interpretation
If the RLU value of the seeded tube is ≥ 30,000, then the sample is not inhibitory to amplification and there apparently was no target available for amplification. If the RLU value of the seeded tube is below 30,000, then the sample is inhibitory to amplification and another sample should be evaluated. If the repeat testing of the unseeded specimen is positive, the MTD test result may be reported as positive. The most likely explanation for this type of result is random sampling; i.e., that the first aliquot did not contain target for amplification, while the second aliquot did. The RLU value of the unseeded tube may be either positive or negative because the aliquot of sediment may or may not contain *M. tuberculosis* rRNA.

4. Laboratory Contamination Monitoring Control

To monitor for laboratory contamination with amplicon or *M. tuberculosis* cells, the following procedure can be performed:

Protocol - Testing the Laboratory for Contamination by Amplicon or *M. tuberculosis* cells.

a. Place 1 mL of sterile water in a clean tube. Wet a sterile polyester or dacron swab with sterile water.
b. Wipe area of bench or equipment to be tested.
c. Place the swab in the water and swirl gently. Remove the swab while expressing it along the side of the tube. Discard the swab into a container containing a 1:1 dilution of household bleach.
d. Add 25 µL of the water containing the expressed swab material into an Amplification Tube containing 25 µL Amplification Reagent and 200 µL Oil Reagent.
e. Follow the Test Procedure for amplification and detection.

Interpretation
If the results are ≥30,000 RLU, the surface is contaminated and should be decontaminated by treating with bleach as recommended in Test Procedure, Equipment Preparation. If contamination of the water bath is suspected, 25 µL of water bath water can be amplified as described for the expressed swab material providing no antimicrobials are used in the water bath.

Sample Preparation

1. Label a sufficient number of Mycobacterium Lysing Tubes to test the samples and 1 each of the Amplification Cell Positive and Negative Controls. Remove and retain the caps.
2. Pipette 200 µL Mycobacterium Specimen Dilution Buffer (1) into all Mycobacterium Lysing Tubes.
3. Transfer 50 µL decontaminated, well-vortexed specimen or cell control from its container to the correspondingly labeled Mycobacterium Lysing Tube.
4. Recap the Mycobacterium Lysing Tubes.
5. Vortex 3 seconds.

Sample Lysis

1. Push the Mycobacterium Lysing Tubes through the Sonicator Rack so that the reaction mixture in the bottom of the tube is submerged but the caps are above water. Place Sonicator Rack on water bath sonicator. **DO NOT ALLOW THE TUBES TO TOUCH THE BOTTOM OR SIDES OF THE SONICATOR.**
2. Sonicate for 15 minutes but no more than 20 minutes. Samples and controls that have been sonicated are now referred to as "lysates".

Amplification

1. Label amplification tubes (12 x 75 mm polypropylene tubes) near the top of the tube with numbers that correspond to those used on the Mycobacterium Lysing Tubes. Also label amplification tubes for each of the Amplification Cell Positive and Negative Controls.
2. Add 25 µL *reconstituted* Mycobacterium Tuberculosis Amplification Reagent to each amplification tube using a repeat pipettor. Add 200 µL Mycobacterium Oil Reagent (3) to each amplification tube using a repeat pipettor.
3. Transfer 50 µL lysate to the bottom of the appropriately labeled amplification tube using a separate extended length hydrophobically plugged pipette tips for each transfer.
4. Incubate the tubes at 95°C for 15 minutes, but no more than 20 minutes, in the dry heat bath.
5. Prepare the enzyme mix by adding 1.4 mL Mycobacterium Enzyme Dilution Buffer (4B) to the lyophilized Mycobacterium Enzyme Reagent (4A). Swirl to mix. Do not vortex.
6. Transfer the tubes to the 42° ± 1°C dry heat bath or water bath and allow them to cool for 5 minutes. **DO NOT ALLOW THE TUBES TO COOL AT ROOM TEMPERATURE. DO NOT COVER THE WATER BATH.**
7. Add 25 µL enzyme mix to each amplification tube using a repeat pipettor while tubes are at 42° ± 1°C. Shake to mix. Incubate at 42°C for 2 hours, but no more than 3 hours. Sealing cards or snap top caps should be used during this incubation step. **DO NOT COVER THE WATER BATH.**

Tubes may be covered and placed at 2°-8°C for up to 2 hours or at -20°C overnight after the 2 hour incubation. If stored at -20°C overnight, tubes must be completely thawed at room temperature or at no greater than 42°C prior to the Termination step. If held overnight, snap caps rather than sealing cards should be used.

Termination

Add 20 µL Mycobacterium Termination Reagent (5) to each tube using a repeat pipettor. Cover tubes with sealing cards or snap top caps. Shake to mix. Incubate at 42°C for 10 minutes.

Tubes may be covered and placed at 2° - 8° C for up to 2 hours or at -20° C overnight after the Termination step. If stored at -20°C overnight, tubes must be completely thawed at room temperature or at no greater than 60°C prior to the Hybridization step.

Hybridization

1. Reconstitute lyophilized Mycobacterium Tuberculosis Probe Reagent (6A) with 6 mL Mycobacterium Hybridization Buffer (6B). Mycobacterium Tuberculosis Probe Reagent (6A) and Mycobacterium Hybridization Buffer (6B) must be at room temperature prior to starting the test. If Mycobacterium

Hybridization Buffer (6B) has been refrigerated, warm at 60°C while swirling gently to ensure that all the components are in solution. Vortex until the solution is clear (this could take up to 1 minute). The reconstituted probe reagent is stable for 1 month at 2°-8°C after reconstitution. If the reconstituted probe has been refrigerated, warm reconstituted probe reagent at 60°C while swirling gently to ensure that all components are in solution.

2. Place 100 µL Mycobacterium Tuberculosis Hybridization Positive Control (HP) and 100 µL Mycobacterium Hybridization Negative Control (HN) into correspondingly labeled 12 x 75 mm polypropylene tubes.
3. Add 100 µL *reconstituted* Mycobacterium Tuberculosis Probe Reagent (6A) to each tube (the HP tube and the HN tube) using a repeat pipettor. Cover tubes with sealing cards. Vortex 3 times for approximately 1 second each time until the reaction mixture is uniformly yellow.
4. Incubate at 60°C for 15 minutes, but no more than 20 minutes, in a dry heat bath or water bath.

Selection

1. Mycobacterium Selection Reagent (7) must be at room temperature prior to starting the test. Remove tubes from the 60°C water bath or dry heat bath and add 300 µL Mycobacterium Selection Reagent (7) using a repeat pipettor. Cover tubes with sealing cards or snap caps. Vortex 3 times for approximately 1 second each time until the reaction mixture is uniformly pink.
2. Incubate tubes at 60°C for 10 minutes, but no more than 11 minutes, in a dry heat bath or water bath.
3. Remove tubes from the water bath or dry heat bath. Cool tubes at room temperature for at least 5 minutes but not more than 1 hour. Remove sealing cards or caps.

Detection

1. Select the appropriate protocol from the menu of the luminometer software. Use a 2 second read time.
2. Using a damp tissue or paper towel, wipe each tube to ensure that no residue is present on the outside of the tube, and insert the tube into the luminometer according to the instrument directions. Tubes must be read within 1 hour of Selection Step 3.
3. When the analysis is complete, remove the tube(s) from the luminometer.
4. After reading the reaction tubes, carefully fill them to the top with a 1:9 dilution of household bleach using a squirt bottle. Allow tubes to sit with bleach for a minimum of 1 hour before discarding. This will help to prevent contamination of the laboratory environment with amplicon.
5. Test tube racks, including racks used for the specimens and tests, should be decontaminated by complete immersion in a 1:1 dilution of household bleach with water for a minimum of 15 minutes. The bleach should then be rinsed off with water and the racks should be allowed to air dry or should be wiped dry.
6. Decontaminate the laboratory surfaces and equipment using a 1:1 dilution of household bleach.

PROCEDURAL NOTES

A. Reagents
   1. Enzyme Reagent should not be held at room temperature for more than 15 minutes after it is reconstituted.
   2. Mycobacterium Hybridization Buffer (6B) may precipitate. Warming and mixing the Mycobacterium Hybridization Buffer (6B) at 60°C will dissolve the precipitate.

B. Temperature
   1. The amplification, hybridization and selection reactions are temperature dependent; ensure that the water bath or dry heat bath is maintained within the specified temperature range.
   2. The tubes must be cooled at 42°C for 5 minutes before addition of enzyme mix for optimal amplification performance.
   3. The temperature is critical for the amplification (42° ± 1°C).

C. Time
   Incubation times as stated in the Test Procedure are important. Follow the time limits specified in the Test Procedure.

D. Water Bath
   1. The level of water in the water bath should be maintained to ensure that the entire liquid reaction volume in the reagent tubes is submerged, but the level must not be so high that water gets into the tubes.
   2. During the amplification step, water bath covers should not be used to ensure that condensate cannot drip into or onto the tubes.

E. Vortexing
   It is important to have a homogeneous mixture during the hybridization and selection steps, specifically after the addition of the *reconstituted* Mycobacterium Tuberculosis Probe Reagent (6A) (the reaction mixture will be uniformly yellow) and Mycobacterium Selection Reagent (7) (the reaction mixture will be uniformly pink).

TEST INTERPRETATION

The results of the MTD test are based on a 30,000 RLU cut-off value. Samples producing signals greater than or equal to the cut off value are considered positive. Samples producing signals less than the cut off value are considered negative.

A. Quality Control Results and Acceptability
   The Amplification Cell Negative Control and Amplification Cell Positive Control should produce the following values:

   Amplification Cell Negative Control < 20,000 RLU
   Amplification Cell Positive Control ≥ 500,000 RLU

   The Hybridization Negative Control and Hybridization Positive Control should produce the following values:

   Hybridization Negative Control < 5,000 RLU
   Hybridization Positive Control ≥ 15,000 RLU

   Patient test results must not be reported if the MTD test control values do not meet the criteria above.

   Target values for amplification cell positive and cell negative controls should be determined in each laboratory using test results for each batch of prepared controls. See NCCLS-recommended procedures for using target values and limits, C24-A, Internal Quality Control Testing: Principles and Definitions[8].

B. Patient Test Results
   If the controls do not yield the expected results, test results on patient specimens in the same run must not be reported.

   A value of ≥ 30,000 RLU is considered positive for *M. tuberculosis* complex rRNA.
   A value of < 30,000 RLU is considered negative for *M. tuberculosis* complex rRNA.

REPORTING OF RESULTS

If the AFB smear and MTD test result are positive, report the following:

> "AFB smear positive and *Mycobacterium tuberculosis* complex rRNA detected. AFB culture pending. Specimen may contain both MOTT and *M. tuberculosis* or *M. tuberculosis* alone."

If the AFB smear is positive and the MTD test is negative, then report the following:

> "AFB smear positive; no *Mycobacterium tuberculosis* complex rRNA detected. AFB culture pending. Specimen may not contain *M. tuberculosis*, or result may be falsely negative due to low numbers of *M. tuberculosis* in the presence or absence of MOTT, or interference with assay detection by specimen inhibitors."

LIMITATIONS

Use only for the detection of members of the *M. tuberculosis* complex using sediments prepared following the NALC/NaOH and NaOH procedures recommended by the CDC[7]. This test may only be used with sediments prepared from sputum (induced or expectorated), tracheal aspirates, or bronchial specimens (e.g. bronchoalveolar lavages and bronchial aspirates).

The MTD test is specific for, but does not differentiate among, members of the *M. tuberculosis* complex, i.e., *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, and *M. microti*. *M. celatum* and *M. terrae*-like organisms will cross react if present at concentrations higher than 30 CFU per test. However, *M. celatum* or *M. terrae*-like organisms are rare clinical isolates.

Test results may be affected by specimen collection and transport, specimen sampling variability, laboratory procedural errors, sample misidentification, and transcriptional errors. A negative test does not exclude the possibility of isolating an *M. tuberculosis* complex organism from the specimen.

TROUBLE SHOOTING

OBSERVATION	POSSIBLE CAUSES	RECOMMENDED ACTIONS
Elevated Amplification Cell Negative Controls (≥ 20,000 RLU)	• Insufficient mixing after addition of the Mycobacterium Selection Reagent (7).	Achieve complete mixing. Visually verify a uniformly pink solution after vortexing.
	• Insufficient care taken during set up of the reactions and the resultant amplification of contaminating materials introduced at that time.	Exercise extreme care when pipetting. The spent reaction tubes must be decontaminated with a 1:9 dilution of household bleach as described in the Test Procedure section. Laboratory bench surfaces, dry heat bath, water baths and pipettors must be decontaminated with a 1:1 dilution of household bleach as described in the Test Procedure. Tubes must be wiped with a damp tissue or paper towel prior to reading in the luminometer.
	• Contamination of lab surface or reagents.	
	• Failure to wipe tubes prior to reading in the luminometer.	
Low Mycobacterium Tuberculosis Amplification Cell Positive Control values (<500,000 RLU)	• Performed the amplification step outside the recommended temperature range.	Check water bath or dry bath temperature and adjust as necessary to achieve the temperature ranges specified in procedure.
	• Insufficient mixing after addition of the reconstituted Mycobacterium Tuberculosis Probe Reagent.	Carefully vortex as specified. (See Hybridization, #1.) Visually verify a clear solution after vortexing.
	• Allowed the Selection step to go over the recommended time limit.	Carefully time the 60°C incubation in the Selection step to be between 10 minutes and 11 minutes.
Elevated Mycobacterium Hybridization Negative Controls (≥ 5,000 RLU)	• Insufficient mixing after the addition of the Mycobacterium Selection Reagent.	Achieve complete mixing. Visually verify a uniformly pink solution after vortexing.
	• Performed the hybridization outside the recommended temperature range.	Check the water bath or dry bath temperature, and adjust as necessary to achieve the temperature ranges specified in procedure.
	• Performed the hybridization for an insufficient length of time.	Carefully time 60°C incubation for at least 15 minutes, but less than 20 minutes.
	• Read the tubes in the luminometer before they have cooled.	Cool tubes at room temperature for at least 5 minutes before reading.
Low Mycobacterium Tuberculosis Hybridization Positive Controls (< 15,000 RLU)	• Allowed the Selection step to go over the recommended time.	Carefully time the 60°C incubation in the Selection step to be between 10 minutes and 11 minutes. Tubes must be wiped with a damp tissue or paper towel prior to reading in the luminometer.

EXPECTED VALUES

A. ***Range of Control Values Observed in the Clinical Studies***

The RLU range for the controls observed in a six site clinical study was:

	RLU (N=243)	
	Range	Mean
Amplification Positive Cell Control	549,000 - 5,000,000*	2,300,000
Amplification Negative Cell Control	1,100 - 17,800**	6,200
Hybridization Positive Control	15,500 - 95,800	49,300
Hybridization Negative Control	400 - 4,100	1,100

* Three results observed between 2,400 - 250,000 RLU

**Three results observed at > 20,000 RLU

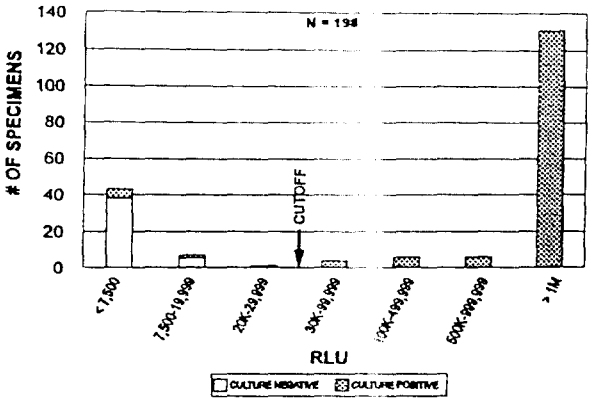
B. **Range of RLU Values for Clinical Specimens**

The range of RLU values for the 147 specimens that were MTD test positive was 32,000 to 3,600,000 RLU. For all of these specimens, *M. tuberculosis* was either cultured from the specimen (138/147) or was cultured from another specimen from the same patient (9/147) collected within the same time frame.

For the 51 MTD test negative specimens, the range of values was 2,200 to 24,000 RLU (7 of these specimens grew *M. tuberculosis* from culture).

The frequency distribution of the RLU values for all these AFB smear positive specimens is shown below.

Distribution of Results From
Untreated Smear Positive Specimens (6 Sites)



Culture Results

M. tuberculosis NEG	38	5	1	0	0	0	0
M. tuberculosis POS	5	2	0	4	6	6	131

PERFORMANCE CHARACTERISTICS

A. Clinical Evaluation

The MTD test was evaluated in studies at 6 sites where AFB smear results and semi-quantitative mycobacterial culture results were documented for 6,079 specimens from 2,609 patients. Of these, 4,000 specimens were collected from 1,898 patients not on antituberculous therapy. The 6 study sites were geographically diverse: 5 were large metropolitan hospital centers with tuberculosis treatment centers and one was a state public health laboratory.

Of the total specimens tested, 198 specimens were AFB smear positive and were from 78 patients not on therapy. Of these, 145 were culture positive for *M. tuberculosis* complex; 2 of these were further identified as *M. bovis*. MTD detected 138 of these culture positive specimens; 1 specimen also grew MOTT in addition to *M. tuberculosis*. Nine (9) additional specimens were MTD positive when the companion culture was negative; however, for these, other cultures from the same patient collected within the same time frame were culture positive.

SMEAR POSITIVE SAMPLES
FROM UNTREATED PATIENTS

MTD vs. Culture (N=198)

		Culture +	Culture -
MTD	+	147	0
	-	7	44

The overall MTD test sensitivity was 95.5% (147/154) and specificity was 100% (44/44) compared to culture results. The ranges at the 6 sites for sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) are also shown in the following table, along with the 95% confidence intervals for the performance estimates:

	Overall Percentage	Number versus Total	Range of Percentages at Six Sites	95% Confidence Interval
Sensitivity	95.5%	147/154	85.7-100%	90.9-98.2%
Specificity	100%	44/44	100%	93.4-100%
PPV	100%	147/147	100%	98.0-100%
NPV	86.3%	44/51	76.0-100%	73.7-94.3%

Of the 7 specimens that were negative with the MTD test but positive for *M. tuberculosis* complex by culture, 2 were from specimens with MOTT/*M. tuberculosis* mixed cultures. Six (6) of the 7 specimens were tested for inhibition using a spike-in procedure with Mtb RNA and frozen reserved sediment; 2 specimens inhibited detection of the RNA.

Of the 44 AFB smear positive specimens that were culture and MTD negative for Mtb complex: 33 specimens grew MOTT on culture, 4 were from patients with other cultures positive for MOTT, 3 were from a single patient with negative mycobacterial cultures but clinically diagnosed reactivated tuberculosis, and 4 were from patients with negative mycobacterial cultures and who were not clinically diagnosed with tuberculosis.

B. Precision Studies

Precision panels, consisting of 2 negative samples, 2 low positive samples (83 CFU/test) and 2 moderately high positive samples (750 CFU/test) were tested at 3 sites. The positive samples were prepared by spiking a processed sediment pool with known amounts of *M. tuberculosis*. The samples were tested in triplicate twice a day for 3 days at the 3 sites. Positive and negative amplification and hybridization controls were included in each run.

Because there was no significant site-to-site or day-to-day variability observed, the data from all 3 sites were combined and are presented below.

Precision Studies

	# Observations	% Correct	Range (RLU)	Mean (RLU)
Sample 1 High Positive	108*	100%	76,000 - 3,400,000	3,000,000
Sample 2 Low Positive	108	100%	1,600,000 - 3,300,000	2,900,000
Sample 3 Negative	108	100%	2,700 - 22,000	4,800
Positive Cell Control	54	100%	2,100,000 - 3,300,000	2,900,000
Negative Cell Control	53**	96.2%	2,900 - 29,000	5,500
HPA Positive Control	18	100%	41,000 - 56,000	49,000
HPA Negative Control	18	100%	770 - 2,100	1,100

* One observation - pipetting error
** One observation not performed

C. Reproducibility

The reproducibility panel consisted of 25 samples with Amplification Negative Controls interspersed between each sample for a total of 50 samples. The Reproducibility Panel was tested at 4 sites.

Overall, 94.1% (113/120) of the negative samples yielded the expected results and 100% (80/80) of the positive samples yielded the expected results. After 2 sites repeated their runs, the overall percentage correct was 97.5% (117/120) for the negative samples and 100% (80/80) for the positive samples.

D. Analytical Specificity

Specificity of the MTD test was assessed using bacteria, fungi, and viruses. For bacteria and fungi, specificity testing included 160 strains (151 species from 58 genera) of closely related mycobacteria, other organisms causing respiratory disease, and normal respiratory flora or organisms representing a cross-section of phylogeny. Typed strains were obtained from the American Type Culture Collection (ATCC), and 5 isolates were obtained from clinical laboratories. Lysates prepared from actively growing cultures (or rRNA in 4 cases) were evaluated in the MTD test according to the Test Procedure. Approximately 2-4 x 10^6 colony forming units (CFU) per reaction were tested. *M. celatum* and some *M. terrae*-like strains will yield positive MTD test results if present at high concentrations. RLU values were negative (4,500-29,000 RLU) at 30 CFU per test. RLU values observed at 3,000 to 30,000 CFU per test were 18,000 to 2,500,000 RLU.

E. Limits of Detection

Thirty (30) strains of *M. tuberculosis* from a wide geographic distribution, including representative drug-resistant and drug-sensitive strains, were detected with the MTD test. The MTD test detected 1 CFU per test of all 30 strains. Strains of the *M. tuberculosis* complex other than *M. tuberculosis* were detected at a range of 2,100,000 to 3,100,000 RLU.

F. Recovery

Five (5) fg *Mycobacterium tuberculosis* rRNA was tested in the presence of approximately 290,000 CFU per test (50 µL) of the following relevant non-target organisms: *Haemophilus influenzae, Streptococcus pneumoniae, Legionella pneumophila, Pseudomonas aeruginosa, Mycobacterium gordonae, M. avium, Nocardia asteroides, N. otitidis-caviarum, Corynebacterium pseudotuberculosis, C. diphtheriae, Gordona sputi,* and *Rhodococcus bronchialis.* All positive test results remained positive even in the presence of as many as 290,000 CFU/test of non-target bacteria.

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